1997

Status Report

Stored-Product Insects

Research of

of Military Importance

Biological Research Unit
U. S. Grain Marketing Research Laboratory
Grain Marketing and Production Research Center

1515 College Avenue
Manhattan, Kansas 66502

Telephone: (785) 776-2705
FAX: (785) 537-5584
Internet Home Page: http://bru.usgmrl.ksu.edu

1997 Status Report

"Stored-Product Entomology Research"

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U. S. GRAIN MARKETING RESEARCH LABORATORY

GRAIN MARKETING AND PRODUCTION RESEARCH CENTER

LABORATORY MISSION

The mission of the U. S. Grain Marketing and Research Laboratory is to develop new knowledge, information, and technologies needed to solve problems associated with the harvest, storage, marketing, and overall quality and end-use properties of cereal grains. The Laboratory program focuses on post-harvest aspects of grain marketing and includes a major commitment to incorporation of optimum end-use quality characteristics into new grain varieties in cooperation with plant breeders. The Laboratory provides multidisciplinary research on these issues by three research units:

Biological Research Unit

Engineering Research Unit

Grain Quality and Structure Research Unit

MISSION OF THE BIOLOGICAL RESEARCH UNIT

The mission of the Biological Research Unit is to develop new, ecologically-based methods to manage insect pests in stored grain, processed commodities and storage and processing facilities. A multi-disciplinary team conducts fundamental and applied research on new biological and ecological approaches to pest management that will reduce the use of traditional pesticides on grain and grain products. Successful completion of the mission will provide a sustainable supply of high-quality cereal products.

We conduct research on biological control agents, insect-resistant packaging, novel physiological control techniques, host plant resistance, insecticide deployment and resistance management strategies, insect biochemistry and genetics, insect population monitoring, population dynamics and behavior, and computer-based integrated pest management systems.

BIOLOGICAL RESEARCH UNIT
GRAIN MARKETING AND PRODUCTION RESEARCH CENTER

STAFFING/AREA OF RESEARCH EMPHASIS

Research Leader

Dr. James E. Throne Supervisory Research Entomologist	Ecology, Modeling, Seed Resistance to Insects

Scientists

Dr. Franklin H. Arthur Research Entomologist	Integrated Pest Management Technologies
Dr. James E. Baker, Research Entomologist	Parasitoid Biology and Toxicology, Digestive Physiology
Dr. Richard W. Beeman Research Entomologist	Genetics/Molecular Biology, Insecticide Resistance
Dr. Alan K. Dowdy Research Entomologist	Insect Ecology/Behavior in Commercial Facilities
Dr. Paul W. Flinn Research Biologist	Modeling/Expert Systems
Dr. David W. Hagstrum Research Entomologist	Insect Ecology, Modeling, Sampling, Acoustic Detection
Dr. Ralph W. Howard Research Chemist	Chemical Ecology, Biological Control
Dr. Donovan E. Johnson Research Microbiologist	Microbiology of Insect Pathogens
Dr. Karl J. Kramer Research Chemist	Insect Biochemistry, Physiology, Biopesticides
Dr. Michael A. Mullen Research Entomologist	Insect Trapping, Insect Resistant Packaging
Dr. Brenda S. Oppert Research Biochemist	Insect Biochemistry, Physiology, Toxicology, *Bacillus thuringiensis* Research

RESEARCH HIGHLIGHTS AND TECHNOLOGY TRANSFER FOR FY97:

INSECTICIDE EFFICACY. Determined that confused flour beetles are more susceptible than red flour beetles to cyfluthrin wettable powder applied on porous concrete. Red flour beetle mortality increased as the application rate and exposure interval increased. (Franklin H. Arthur)

SHOWED THAT MORTALITY of the 5th instar indianmeal moths exposed to cyfluthrin wettable powder on concrete increased as the application rate increased, but that there was no difference in either pupation or adult emergence when larvae were exposed for short intervals of 0.5 to 4 hours. After 3-6 weeks, adult emergence exceeded 20% at all application rates. (Franklin H. Arthur)

AERATION OF GRAIN could be used to manage farm-stored corn in the southern United States, but the threshold temperatures used for activating the aeration fans would have to be higher in those areas where there is an extended warm fall season. Increasing the fan speed may also be necessary to cool the corn quicker. (Franklin H. Arthur)

ASSISTED GUSTAFSON, INC., on the residual activity of cyfluthrin applied to concrete. (Franklin H. Arthur)

COLLABORATED WITH INDUSTRIAL FUMIGANTS AND WHITMIRE, INC., to evaluate a new encapsulated formulation of cyfluthrin as a wheat protectant. (Franklin H. Arthur)

COLLABORATED WITH RHONE-POULANC TO EVALUATE AN INSECTICIDE that is a member of a new class of chemicals with a novel mode of action. (Franklin H. Arthur/Richard W. Beeman)

COLLABORATED WITH MYCOTECH CORP. TO EVALUATE A STRAIN OF ENTOMOPATHIC FUNGI for use on raw grains and flooring surfaces. (Franklin H. Arthur)

COLLABORATED WITH KANSAS STATE UNIVERSITY, DEPT. OF GRAIN SCIENCE, ON AERATION STRATEGIES for farm-stored corn. (Franklin H. Arthur)

SERVED ON THE PLANNING COMMITTEE OF THE 5TH NATIONAL STORED PRODUCT IPM TRAINING CONFERENCE, PURDUE UNIVERSITY. (Franklin H. Arthur/Alan K. Dowdy)

ASSISTED THE DEPARTMENT OF GRAIN SCIENCE AT KANSAS STATE UNIVERSITY in tests of the efficacy of a dichlorvos aerosol application and a heat treatment in the flour mill.
(Franklin H. Arthur/Alan K. Dowdy/Michael A. Mullen)

DEMONSTRATED THE STABILITY OF MALATHION RESISTANCE in populations of *Anisopteromalus calandrae,* a beneficial parasite of stored-grain pests. (James E. Baker)

CONSULTED WITH BIOFAC CROP CARE, MATHIS, TEXAS, about the use of parasitic Hymenoptera to control infestations in peanut warehouses.
(James E. Baker)

COLLABORATED WITH THERMO TRILOGY CORP., Columbia, MD, about testing neem products for storage pest control. (James E. Baker)

PROVIDED PARASITIC WASPS FOR PEST CONTROL IN A LOCAL GROCERY STORE. (James E. Baker/Michael A. Mullen/Alan K. Dowdy)

DEVELOPED A LABORATORY SELECTION METHOD for increasing the frequency of insecticide resistance in the beneficial parasitic wasp, *Bracon hebetor*. (James E. Baker)

CLONED AND SEQUENCED ESTERASE cDNAS from malathion resistant and susceptible strains of a beneficial parasitic wasp, *Anisopteromalus calandrae.* DNA markers that differentiate genetic differences between the resistant and susceptible strains have been developed. (James E. Baker/Alan K. Dowdy)

GENOME MAP FOR STORED-PRODUCT INSECT PEST. Constructed a complete genome molecular map of the red flour beetle using randomly amplified polymorphic (RAPD) DNA markers, visible mutant markers, and specific gene markers. The map is now being used to facilitate map-base cloning of important insect control genes. (Richard W. Beeman)

GENE VECTORS FOR GENETIC ENGINEERING OF INSECTS. Developed gene vectors for transferring insects with novel genes. Recent improvements in transformant selection technology offer hope that potentially-useful transposon vectors will be used to genetically manipulate stored-grain insect genomes.
(Richard W. Beeman)

MECHANISMS OF HYBRID INVIABILITY ELUCIDATED. Demonstrated that killer genes known as "Medea" (M) factors are required for larval mortality associated with a hybrid inviability gene (H). Knowledge of the detailed mechanisms of larval kill in the M and H systems could be exploited in new, naturally-based insect control strategies. (Richard W. Beeman)

DEVELOPED A LABORATORY TECHNIQUE BASED ON THE POLYMERASE CHAIN REACTION (PCR) for cloning insertions of foreign DNA into insect chromosomes and provided advice and materials to several university and government research laboratories that now use this technique. (Richard W. Beeman)

SCREENING OF THE INDIANMEAL MOTH FOR GENETIC FINGERPRINTS linked to resistance to the bacterial insecticide *Bacillus thuringiensis* was conducted. The messenger RNA that codes for aminopeptidase in Indianmeal moth was characterized. Screening of *Anisopteromalus calandrae*, a parasitoid of the rice weevil, for genetic fingerprints linked to resistance to Malathion. We characterized the expression of messenger RNA that codes for a carboxyesterase in *A. calandrae*. (Alan K. Dowdy)

HEAT STERILIZATION PROCEDURES FOR INSECT MANAGEMENT were monitored in three processing facilities. The distribution and stratification of heat has an impact on the effectiveness of this method for insect control. Most areas examined would benefit from the addition of fans to improve air circulation that will result in more uniform application of heat. (Alan K. Dowdy)

EVALUATE THE USE OF HEAT AND DIATOMACEOUS EARTH on insect mortality. Laboratory tests indicate that the use of diatomaceous earth in combination with heat has the potential of reducing the temperature or time necessary to effect adequate insect management. When red flour beetles were treated with diatomaceous earth and exposed to 50°C for 15 minutes, mortality was comparable to beetles that were heated to 50°C for 30 minutes but not treated with diatomaceous earth. (Alan K. Dowdy)

SPATIAL ANALYSIS METHODS. The locations of stored-product insect infestations were identified using spatial analysis methods. Sources of infestations previously unknown to facility managers were identified. Insect infestations appear to be related to equipment design problems, inadequate sanitation and poor stock rotation. (Alan K. Dowdy)

USED A SPATIAL MODEL OF RUSTY GRAIN BEETLE DENSITY and bin temperature to simulate effects of time of aeration, bin size, and latitude on insect populations in stored wheat. Starting automatic aeration controllers at harvest suppressed insects below economic levels until the spring. (Paul W. Flinn/David W. Hagstrum)

MODIFIED THE MODELS IN STORED GRAIN ADVISOR (SGA) to predict effects of using automatic aeration controllers starting at harvest. SGA is presently available to the public through the extension services of Oklahoma State University, Kansas State University and Montana State University, and over 1000 copies have been distributed to customers. (Paul W. Flinn/David W. Hagstrum)

DEVELOPED A COMPUTER MODEL THAT PREDICTS THE EFFECTS OF LOW OXYGEN ATMOSPHERES on rice weevil populations in stored grain. This model can predict the duration of fumigation using low oxygen levels required to produce a given mortality and it can be used to predict insect density in grain 1-2 months post-fumigation. (Paul W. Flinn/David W. Hagstrum)

INSECT RESPONSES TO GRAIN TEMPERATURE CHANGES. Discovered that rusty grain beetles move toward warmer-temperature grain, even in very small temperature gradients. Thus, beetles will move toward the center of the grain mass as the grain cools in the fall. (Paul W. Flinn/David W. Hagstrum)

PREDICTING INSECT DENSITY FROM PROBE TRAP CATCH IN FARM-STORED WHEAT. Improved methods were developed for estimating actual insect density from trap catch using equations that adjust for the effects of grain temperature on trap catch. These methods will make probe traps more useful as monitoring tools for stored-grain insect pest management programs. (David W. Hagstrum/Paul W. Flinn)

AREA-WIDE IPM FOR SUPPRESSION OF INSECT PESTS IN STORED WHEAT. A 5-year Area-Wide IPM project was recently funded by ARS to determine whether more uniform application of insect pest management across the marketing system could reduce insect problems in stored wheat. The program should reduce the frequency of pesticide application, the cost of pest management and the risk of insect problems. (David W. Hagstrum/Paul W. Flinn/ James E. Throne, Frank H. Arthur/Alan K. Dowdy/Michael A. Mullen)

SAMPLING PROGRAMS FOR STORED-PRODUCT INSECTS. Developed a generic method for designing sampling programs for stored-product insects and demonstrated its applicability to many different types of commodity storage

situations. This method will improve the quality of sampling programs and reduce the cost of developing new sampling programs.
(David W. Hagstrum/Paul W. Flinn)

PARASITOID SEX PHEROMONES DISCOVERED. The parasitoids *Cephalonomia tarsalis* and *Pteromalus cereallellae* were shown to use female-produced sex pheromones to attract males of their species. (Ralph W. Howard)

INSECT RESPONSES TO BACTERIAL INVASION. Demonstrated that essential fatty acids (eicosanoids), which regulate insect immunity to bacterial infections, vary with developmental life stage. These findings suggest that control of insect pests with pathogens would be more effective if treatments were timed to occur when pest populations had their lowest level of essential fatty acids. (Ralph W. Howard)

DEMONSTRATED THAT THE ABILITY OF AN INSECT TO FIGHT BACTERIAL INFECTIONS by forming nodules is directly correlated with the insect's age, the species of invading pathogen, and the number of pathogenic cells invading the insect's body. (Ralph W. Howard)

BEHAVIOR OF PARASITES OF STORED GRAIN PESTS. Demonstrated that the parasitic wasp, *Cephalonomia tarsalis,* has a complex behavioral pattern that it uses to locate, recognize, and paralyze its host, the saw-toothed grain beetle. This information will be used to develop improved biological control strategies that use this parasitoid. (Ralph W. Howard)

B. THURINGIENSIS SPORES AND CRYSTALS ARE NECESSARY FOR MAXIMUM INSECT TOXICITY. Spore coat protein can enhance (synergize) crystal protein and thus the combination of spores and crystals provide much more effective control of the Indianmeal moth (*Plodia interpunctella*) than each one can separately. (Donovan E. Johnson)

KNOWLEDGE OF NOVEL INSECT ENZYME GENES MAY LEAD TO MORE EFFECTIVE PEST MANAGEMENT. Certain enzymes that are selectively toxic to insects are being developed by ARS as biopesticides for insect control purposes. A gene for an insect molting enzyme has been cloned and characterized by ARS and Kansas State University scientists. This enzyme degrades the protective linings of the insect's gut and exoskeleton and is toxic when fed to insects. A seed company has been licensed by ARS and KSU to evaluate this gene for resistance to pest insects when it is expressed by transgenic plants. This collaboration is a critical step in the commercial development of this transgene as a biopesticide for many types of agricultural pest insects. (Karl J. Kramer)

PATENT APPLICATIONS FOR THE USE OF INSECT CHITINASE AS A BIOPESTICIDE. ARS scientists at Manhattan, Kansas, in cooperation with scientists at Kansas State University, are working to enhance the resistance of plants to insects using chemical defense transgenes. One of the genes is an insect molting enzyme gene that can be manipulated by the agricultural biotechnology industry for the improvement of host plant resistance to insect pests. Efforts to obtain US and international patents for the use of insect chitinase as a biocide in the United States and several foreign countries are in progress. (Karl J. Kramer)

KNOWLEDGE OF INSECT SKELETAL STRUCTURES MAY LEAD TO MORE EFFECTIVE PEST MANAGEMENT. The insect exoskeleton is a good target for novel pest management strategies because of the unique insect-specific chemistry that occurs during its formation. Development of exoskeleton-targeted insect control agents has been hampered by a lack of basic knowledge about insect skeletal structure and metabolism. ARS and university scientists have identified novel metabolic reactions in insects that help to form and stabilize the exoskeleton. Inhibition of these reactions by biopesticides may be an environmentally-safe method of insect pest control. (Karl J. Kramer)

SERVED IN ADVISORY POSITIONS FOR THE STORED PRODUCTS COMMITTEE OF THE ARMED FORCES PEST MANAGEMENT BOARD. (Michael A. Mullen/Franklin H. Arthur/Alan K. Dowdy)

INSECT RESISTANT PACKAGING. Assisted in development of insect resistant packages for various food packaging companies which have led to significant reductions in insect related complaints. (Michael A. Mullen)

COLLABORATED WITH TENNECO, INC. AND INTERNATIONAL PAPER COMPANY to develop insect repellent materials for use on consumer packages. One repellent has been submitted to FDA for approval. (Michael A. Mullen)

COLLABORATED WITH CONTINENTAL EXTRUSION to design and test a new insect resistant package for processed food products. (Michael A. Mullen)

PHEROMONE TRAPS FOR STORED-GRAIN INSECTS. Continued development of monitoring systems and pheromone-baited traps for use in processing plants, warehouses, and retail outlets. (Michael A. Mullen)

DEVELOPED NEW PHEROMONE TRAP for the Indianmeal moth which can be used in public areas but be hidden from view.
(Michael A. Mullen/Alan K. Dowdy)

AN ECONOMICAL SUBSTRATE ASSAY FOR PROTEINASES IN MIXTURES WAS DEVELOPED. This assay allows the quick determination of the number and types of enzymes without prior purification. (Brenda Oppert)

MANY PRESENTATIONS WERE MADE TO ACADEMIA AND INDUSTRY DESCRIBING PROTEINASE-MEDIATED RESISTANCE TO BT TOXINS. With increased planting of Bt transgenic crops, resistance becomes more of a threat. Seed companies and farmers can combat resistance more effectively if they understand how insects adapt to Bt toxins. (Brenda Oppert)

NEW MECHANISM OF INSECT RESISTANCE TO BIOPESTICIDE DETERMINED. A proteinase-mediated mechanism of insect resistance to the entomocidal toxins of Bacillus thuringiensis has been identified and characterized. Resistant insects lack a proteolytic enzyme that is critical for activation of the precursor forms of the toxins. This knowledge will be helpful in developing stategies for managing resistance to biopesticides in the field.
(Brenda Oppert/Karl J. Kramer)

DEVELOPED A COMPUTER MODEL FOR SIMULATING THE POPULATION DYNAMICS of the almond moth. Validated model for corn, peanuts, and dried citrus pulp. (James E. Throne)

DEVELOPED THE USE OF NEAR-INFRARED REFLECTANCE SPECTROSCOPY for detection of insect larvae in grain kernels, for disinfestation of insect-infested grain, and for identification of insects.
(James E. Throne/ James E. Baker)

DEVELOPED AND VALIDATED A COMPUTER MODEL FOR SIMULATING POPULATION DYNAMICS OF THE PREDATOR, *LYCTOCORIS CAMPESTRIS*. (James E. Throne)

IDENTIFIED COMMERCIAL CORN HYBRIDS that have resistance to maize weevils. (James E. Throne)

TRANSFERRED A COMPUTER MODEL TO A PRIVATE CONSULTANT working with another ARS lab for modification for simulating population dynamics of other insect pests. (James E. Throne)

TRANSFERRED THE ALMOND MOTH MODEL TO A PRIVATE CONSULTANT working with the American Cocoa Research Institute for use in stored cocoa. (James E. Throne)

PROVIDED COPIES OF AND INSTRUCTIONS FOR USING A PROBIT-TYPE ANALYSIS PROGRAM to over twenty scientists at government laboratories and universities in twelve countries. Met with the Director of Research, National Center for Entomology at La Cruz, Chile, to develop use of the program in research in Chile. (James E. Throne)

REVIEW ARTICLES ON APPLICATIONS OF BIOTECHNOLOGY FOR PEST INSECT BIOLOGY RESEARCH AND CONTROL. Unit scientists authored several chapters on the use of biopesticides for insect pest management in several books and journals. These chapters will assist other scientists in their insect research projects and also grain managers and pest control operators in controlling pest insects. (BRU Scientists)

ENTOMOLOGICAL RESEARCH JOURNALS EDITED BY UNIT SCIENTISTS. Several unit scientists served on the editorial boards of entomological journals (Insect Biochemistry and Molecular Biology, Journal of Economic Entomology, Journal of Entomological Science) where they evaluated and edited manuscripts submitted for publication. (BRU Scientists)

RESEARCH PROGRESS AND PLANS

CRIS No. 5430-43000-014-00D

Title: Ecology, Modeling, and Integrated Management of Stored-Product Insects

Main Objectives:

Pest management decisions can be improved by developing better insect monitoring programs and models for predicting insect population growth. Research will provide the technology that grain managers need to detect insect infestations more accurately and earlier, predict insect population growth rates and effects of different control measures, and forecast when insect control will be needed. These management tools are needed for a transition to less chemically dependent integrated pest management programs. This project focuses on acquiring essential biological and ecological data, using these data to develop predictive models of insect population growth, and developing an expert system for stored grain management that uses the models. Studies will expand the range of species and conditions over which existing models predict insect population growth and the range of problems for which the expert system can make pest management recommendations. Expansions will include additional pest species, natural enemies, grain varieties, aeration, cold temperature survival, and insect movement. Improved sampling programs will be developed to collect ecological data and acquire information needed to make pest management decisions. Automatic insect monitoring using acoustical sensors will be investigated.

Investigators: **David W. Hagstrum, James E. Throne, Paul W. Flinn**

1. Specific Objective: Quantify effects of temperature and moisture on fecundity of maize weevils for inclusion in maize weevil model.

Progress FY97: Studies to develop antibody method for quantification of weevil egg plugs have been completed, and manuscript is in preparation.

Plan of Work FY98: Complete manuscript. No further studies planned at this time.

2. Specific Objective: Develop computer model for simulating population dynamics of the predator *Lyctocoris campestris*.

Progress FY97: Model was modified to accurately simulate population dynamics observed in validation study. Manuscript is in preparation.

Plan of Work FY98: Complete manuscript. No further studies planned at this time.

3. **Specific Objective:** Determine the effects of early aeration to cool grain on the efficacy of *Choetospila elegans* for controlling lesser grain borer in wheat at 32 and 25°C.

Progress FY97: Laboratory studies were conducted to assess the effectiveness of the parasitoid wasp, *Choetospila elegans* for controlling lesser grain borer in wheat at 32 and 25°C. The two temperature regimes were used to simulate an unaerated bin of wheat and a bin aerated at harvest time. Suppression of lesser grain borer populations by the parasitic wasps was much greater at 25 than at 32°C. At 25°C, the wasps were able to locate and parasitize most of the larvae that were produced by the adult beetles. This resulted in a very high level of population suppression (99% in comparison to the control at 25°C). In contrast, at 32°C, beetle suppression was only 50% in comparison to the controls. This research demonstrated that biological control using parasitic wasps is ten times more effective when combined with early aeration to cool the grain to 25°C or less. The combination of aeration plus cool temperature resulted in 99% beetle control for over 160 days of storage. This level of control is at least as good as traditional chemical controls for suppressing lesser grain borer in stored wheat, and has none of the environmental hazards associated with chemical control.

Plan of Work FY98: Continue evaluation of other parasitic wasps to determine if they are also more effective in suppressing beetle populations at cooler temperatures.

4. **Specific Objective:** Determine the effects of temperature gradients in stored wheat on the movement and distribution of the rusty grain beetle.

Progress FY97: The rusty grain beetle, *Cryptolestes ferrugineus*, is the most common insect pest of stored wheat in the United States and Canada. Adults and larvae feed mostly on the wheat germ and cause considerable damage. Beetle population growth rate is primarily affected by grain temperature. In the autumn, the periphery of the grain mass cools more rapidly than the center. Beetle populations may be higher in the center if they are able to move from the cool periphery toward the warm center of a grain mass. Temperature gradients were established in a 56 cm diameter cylinder with 9 cm high sides filled with 19.9 kg of hard red winter wheat to determine if the rusty grain beetle would

disperse to warmer areas. Results showed that the rusty grain beetle moved into and remained in warmer areas of the grain mass after 24 hours. Beetles moved into the warmest area of the grain mass even when this area was only 1°C warmer. This study suggests that the rusty grain beetle should move toward the warmer center region of a grain mass as the periphery of the grain cools in the fall. This would allow rusty grain beetle populations to continue to increase in the center of the grain mass during the winter months in bins that were not cooled with aeration fans. The movement toward warmer regions could accelerate the population growth rate of rusty grain beetles in unaerated grain bins. The center of a large bin of unaerated grain will often remain warm during winter months, which allows insect populations to continue to develop and reproduce. Cooling the grain with aeration should greatly reduce rusty grain beetle population growth because of all the grain could be cooled to a temperature below which population growth would occur.

Plan of Work FY98: Continue evaluation of other stored grain beetles to determine if they also move toward warmer areas of the grain mass.

5. **Specific Objective**: Examine the effects of latitude, bin size, and aeration on insect density in stored wheat and determine optimal aeration strategies to prevent insect infestation.

Progress FY97: Computer simulation studies were conducted with a two-dimensional grain bin model. The model was used to simulate the effects of latitude, bin size, and aeration on insect density in stored wheat. The simulations showed that insect density was highest in the center top portion of the grain mass. This also coincided with the region of the grain that remained warmer longest in the fall and winter. Insect density was much higher in wheat stored in Oklahoma than in Kansas or South Dakota, and was also higher in larger than in smaller grain bins. Automatic aeration controllers (fans turned on when outside air temperature was 10°C lower than grain temperature) suppressed insect population growth better than manual aeration starting in November. Automatic aeration also worked better when started at harvest rather than waiting until September. This study showed that when automatic aeration controllers were used to reduce grain temperature as quickly as possible, insect populations were suppressed below economically damaging levels from harvest until the following spring. In most of the U.S., the use of inexpensive automatic aeration controllers should greatly reduce the need for insecticides in stored grain.

Plan of Work FY98: Rewrite the Stored Grain Advisor expert system to include the two-dimensional grain bin model.

6. **Specific objective**: Determine use of near-infrared reflectance spectroscopy for detection of insect in grain kernels.

Progress FY97: We were able to detect lesser grain borers, rice weevils, and Angoumois grain moths in wheat kernels using an automated single-kernel grain characterization system coupled with an NIR system. Grain moisture content, protein content, and wheat class did not affect detection accuracy.

Plan of Work FY98: Conduct studies to determine chemical basis for method.

7. **Specific objective**: Determine use of near-infrared reflectance spectroscopy for killing insects in grain kernels.

Progress FY97: We achieved complete mortality of rice weevils in wheat kernels after 90 sec exposure to NIR in an automated unit. The method provides a safe and inexpensive method for disinfestation of insect-infested grain.

Plan of Work FY98: Conduct further studies to refine method.

8. **Specific objective**: Determine use of near-infrared reflectance spectroscopy for classification of insects and other organisms.

Progress FY97: We were able to classify 11 species of stored-product insects to genus with over 98% accuracy using NIR. The method has potential for classification of any living or dead organism, and has potential for automated identification of insects and other organisms in traps.

Plan of Work FY98: Conduct further studies to refine the method and develop use for other species.

9. **Specific objective**: Determine resistance of commercial corn hybrids to maize weevils.

Progress FY97: Found large differences in number of progeny produced, development time, and progeny weight of weevils developing on commercial corn hybrids.

Plan of Work FY98: Determine chemistry of corn kernels, and correlate with biological parameters.

10. <u>Specific Objective</u>: Develop computer model for simulating population dynamics of the almond moth developing on stored-products.

<u>Progress FY97</u>: Developed and validated a computer model for accurately simulating population dynamics of almond moths in stored corn, peanuts, and dried citrus pulp.

<u>Plan of Work FY98</u>: Manuscript has been submitted to journal. No further studies planned at this time.

11. <u>Specific Objective</u>: Predict insect density from probe trap catch in farm-stored wheat.

<u>Progress FY97:</u> Insect monitoring is important in determining whether insect control is needed and whether pest control was effective. Traps are often easier to use than other methods of estimating insect density. However, the seasonal changes in temperature and other factors can influence trap catch more than the density estimates made with other methods, and methods must be developed for converting trap catch to insect density per volume of grain. We investigated the influence of the seasonal changes in grain temperature in 2 bins of stored wheat on each of 2 farms in Kansas on the prediction of insect density from probe trap catch. Estimates of insect density based upon the numbers of adult insects caught using probe traps differed from those based upon the number of insects found in grain samples. The numbers of insects captured in traps decreased as grain temperature decreased during the storage period even though grain samples indicated that insect populations were still growing. Thus, trap catches did not estimate insect population density consistently throughout the storage period. Therefore, the insect density in grain samples should be estimated from trap catch using the equations that we developed to adjust for the effects of temperature on trap catch. These methods will make probe traps more useful as monitoring tools for stored-grain insect pest management programs.

<u>Plan of Work FY98</u>: Field studies will be conducted to increase our understanding of the phenology of infestation of newly harvested wheat.

12. <u>Specific Objective</u>: Implement an area-wide IPM program for suppression of insect pests in stored wheat.

<u>Progress FY97:</u> A 5-year Area-Wide IPM project was recently funded by ARS to determine whether more uniform application of insect pest management across the marketing system could reduce insect problems in stored wheat. Insects are moved along with grain from one elevator to another. If not controlled, insect problems can be spread from one elevator to another. The first year of the project

will be for planning. The project is a collaboration of the Grain Marketing and Production Research Center, Kansas State University and Oklahoma State University. The objective of the Area-Wide IPM project is to implement a state-of-the-art stored-wheat IPM program in elevator networks in Kansas and Oklahoma. The program will emphasize reducing pesticide use, the cost of pest management and the risk of insect problems. Methods to meet these objectives are intensive insect monitoring, computer models predicting insect population growth and when insect control is needed, sanitation, and early aeration to cool grain and reduce insect and mold population growth. Wheat should be fumigated only when biologically and physically-based methods fail to keep insects below economically damaging levels. Recent changes in grain marketing practices may have changed pest management needs. The project will determine whether current pest management programs are the most cost effective in reducing the risk of insect infestation.

Plan of Work FY98: The planning will be completed and the project will be ready for full implementation by April 1998.

Publications:

Arbogast, R. T., and J. E. Throne. 1997. Insect infestation of farm-stored maize in South Carolina: towards characterization of a habitat. J. Stored Prod. Res. 33: 187-198.

Baker, J. E., R. W. Beeman, J. E. Throne, and J. Perez-Mendoza. 1997. Fitness of an insecticide-resistant parasitic wasp. Resistant Pest Management Newsletter 9: 12-14.

Burks, C. S., D. W. Hagstrum, K. E. Hampton, and A. B. Broce. 1997. Crystallization temperature and chilling injury during overwintering in a feral face fly (Diptera: Muscidae) population. Environ. Entomol. 26:1124-1130.

Dowell, F. E., C. R. Martin, J. L. Steele, D. Wang, J. E. Baker, J. E. Throne, and S. R. Delwiche. 1997. Automated single wheat kernel quality measurement using near-infrared reflectance. ASAE Paper 973022, 9 pages.

Flinn, P. W., and D. W. Hagstrum. 1997. Simulation of the effects of latitude, bin size, and aeration on insect populations in stored wheat. J. Econ. Entomol. 90: 646-651.

Flinn, P. W., and D. W. Hagstrum. Simulation model of low oxygen atmospheres on insect population dynamics in stored grain. Proc. Intern. CAF Conference, Nicosia, Cyprus, April 21-26, 1996. (In press)

Flinn, P. W., and D. W. Hagstrum. Distribution of *Cryptolestes ferrugineus* (Coleoptera: Cucujidae) in response to temperature gradients in stored wheat. J Stored. Prod. Res. (In press)

Flinn, P. W. Effect of temperature on efficacy of *Choetospila elegans* (Hymenoptera: Pteromalidae) to suppress *Rhyzopertha dominica* (Coleoptera: Bostrichidae) in stored wheat. (In press)

Hagstrum, D. W., P. W. Flinn, and J. Gaffney. Response of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) to temperature gradient in stored wheat. J. Econ. Entomol. (In press)

Hagstrum, D. W., Bh. Subramanyam, and P. W. Flinn. Nonlinearity of a generic variance-mean equation for stored grain insect sampling data. Environ. Entomol. (In press)

Hickling, R., W. Wei and D. W. Hagstrum. 1997. Studies of sound transmission in various types of stored grain for acoustic detection of insects. Appl. Acoustics 50: 263-278.

Maier, D. E., W. H. Adams, J. E. Throne, and L. J. Mason. 1996. Temperature management of the maize weevil, *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae) in three locations in the United States. J. Stored Prod. Res. 32: 255-273.

Subramanyam, Bh., D. W. Hagstrum, R. L. Meagher, E. Burkness, W. D. Hutchinson, and S. E. Naranjo. Development and evaluation of sequential sampling plans for *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Cucujidae) infesting farm-stored wheat. J. Stored Prod. Res. (In press)

Subramanyam, Bh., D. W. Hagstrum, R. L. Meagher, E. Burkness, W. D. Hutchinson, and S. E. Naranjo. Sampling to determine treatment efficacy: development and evaluation of enumerative and binomial sampling plans for *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Cucujidae) infesting farm-stored wheat. Proc. Intern. CAF Conference, Nicosia, Cyprus, April 21-26, 1996. (In press)

Throne, J. E., D. W. Hagstrum, and J. Nawrot. A computer model for simulating almond moth (Lepidoptera: Pyralidae) population dynamics. Environ. Entomol. (In press)

RESEARCH PROGRESS AND PLAN

CRIS No. 5430-43000-015-00D

Title: Biological Control of Stored Product Insects With Parasites, Predators and Entomopathogens

Main Objectives:

The goal of this project is to develop economically viable pest management systems that capitalize on desirable biochemical, behavioral and biological traits of parasites, predators and entomopathogens of stored grain insect pests. Specific objectives include characterizing life histories of promising parasites and predators exposed singly and in combination to single- and multiple-species combinations of stored product pests; identifying the semiochemical and behavioral mechanisms used by these biological control agents to locate, recognize and kill their hosts; characterizing behavioral and semiochemical responses by pests that ameliorate the effectiveness of biological control agents; assessing specific stress factors such as temperature, relative humidity, desiccants and inhibitors of insect-specific hormones that might weaken pests and make them more susceptible to biocontrol agents; determining parasite-host release ratios, timing of releases, and effect of multiple-species releases on control efficacy; evaluating possible detrimental effects of releasing biological control agents into stored grain commodities; elucidating the biochemical mechanisms by which the toxins of *Bacillus thuringiensis* (*B.t.*) kill stored product pests, with particular emphasis on the physiology of membrane receptor-protein interactions; evaluating the extent of resistance and cross-resistance to native, cloned, and truncated *B.t.* toxins in stored product insect populations; and characterizing insect resistance mechanisms involving the interaction of *B.t.* toxin proteins with midgut proteinases in *Plodia interpunctella*.

Investigators: **Ralph W. Howard, Donovan E. Johnson, and Brenda S. Oppert**

1. <u>**Specific Objective**</u>**:** Determine the mechanisms by which *Cephalonomia waterstoni* and *C. tarsalis* recognize their specific hosts, the rusty grain beetle and the saw-toothed grain beetle, respectively.

<u>Progress FY97</u>: Conducted detailed behavioral analyses and generated an ethogram for host recognition by *C. tarsalis*. Manuscript is now in preparation.

Plan of Work FY98: Continue with development of ethograms for *C. waterstoni*. Publish the initial results of the study with *C. tarsalis*, and continue development of bioassays for chemical cues used in host recognition.

2. **Specific Objective**: Evaluate the possible roles of pheromones in sexual behavior by the parasitoids *Cephalonomia tarsalis* and *Pteromalus cerealellae.*

Progress FY97: Video protocols were established for generating ethograms, preliminary behavioral data were gathered, and preliminary bioassays were developed.

Plan of Work FY98: Conduct detailed behavioral analyses of the courtship and copulatory behavior of the parasites. Using the resulting ethograms, develop bioassays to separate chemical from other cues. Begin characterization of relevant life history parameters that influence pheromone biology.

3. **Specific Objective:** Characterize the cuticular hydrocarbons of the parasitoid *Pteromalus cerealellae* and determine their semiochemical functions.

Progress FY97: Hydrocarbons have been isolated and identified from male and female parasitoids and percent compositions calculated. In addition, hydrocarbons of two larval hosts of this parasitoid have been identified and quantified. There are striking gender based differences between the parasitoid males and females, and striking similarities between the host larval hydrocarbon profiles.

Plan of Work FY98: Complete identification of all hydrocarbons. Complete statistical analyses of compositional differences, and develop bioassays to characterize semiochemical functions of the hydrocarbons. Begin writing manuscript.

4. **Specific Objective**: Identify cues by which parasitoids locate grain stores containing stored product insects.

Progress FY97: A wind tunnel bioassay chamber was further tested in preliminary experiments using the parasite *C. tarsalis* and its host, the saw-toothed grain beetle.

Plan of Work FY98: Construct a wind tunnel bioassay chamber specific for parasitoids and begin experiments with four stored product parasitoids, assessing their orientation behavior towards odor volatiles from stored grain and stored grain infested with storage pests.

5. **Specific Objective:** Determine effects of spores on entomocidal activity of CryIAb and CryIAc crystal proteins from *B. thuringiensis* toward the Indianmeal moth.

Progress FY97: Spores from *B. thuringiensis* subsp. *kurstaki* and *entomocidus* synergized crystal protein toxicity for larvae of the Indianmeal moth (*Plodia interpunctella*). Preparations of spore-crystal mixtures of either species were more toxic for the larvae than either purified spores or crystals alone (based on dry weight). Spores lost 43% of their toxicity for the Indianmeal moth after 2 hours of UV-irradiation, but remained partially toxic even after 4 hours of continual irradiation. Spore coat protein was toxic for the Indianmeal moth and was synergistic with *B. thuringiensis* subsp. *kurstaki* HD1 crystal protein. Enhanced toxicity of the combined spore-crystal preparation was attributed to a combination of crystal and spore coat protein, and included the effects of spore germination and resulting septicemia in the larval hemolymph. Ultraviolet irradiation of spores reduced the toxicity from septicemia but not the synergism caused by spore coat protein. The potencies of spore-crystal preparations must be carefully evaluated based on contributions from all three factors.

Plan of Work FY98: Continue to characterize synergism of spore coat extracts with crystal protein. Use spore extracts from *B. cereus* and/or spores from a cry-minus *B. thuringiensis* to determine if synergism is real, or if toxicity is merely additive. Further characterize synergism between spore extracts and Cry1Aa, Cry1Ab, and Cry1Ac protein for the Indianmeal moth.

6. **Specific Objective:** Determine membrane binding characteristics of susceptible and *B. thuringiensis* resistant Indianmeal moth for crystal proteins from *B. thuringiensis*.

Progress FY97: Two procedures were discovered that affect the level of detection of binding proteins in brush border membrane vesicles (BBMV). One is the method of membrane disruption; a non-ionic detergent conserves micelle formation and leads to improved toxin protein binding, and the second involves exposure of BBMV to toxin proteins prior to disruption of the membrane vesicles that seems to improve specific protein binding.

Plan of Work FY98: Compare membrane binding proteins from susceptible and resistant Indianmeal moth larvae to specific Cry toxin proteins. Determine resistance to a particular Cry toxin protein correlated with the presence or absence of a specific binding protein.

7. <u>Specific Objective:</u> Investigate the involvement of insect gut proteinases in the development of resistance to insecticidal toxins from *Bacillus thuringiensis* (Bacillus thuringiensis).

<u>Progress FY97:</u> Bacillus thuringiensis susceptible and resistant strains of *P. interpunctella* were analyzed for specific proteinase activities, and it was discovered that certain Bacillus thuringiensis resistant strains lack a critical gut proteinase. This proteinase was demonstrated to hydrolyze Bacillus thuringiensis protoxin. Genetic crosses linked the absence of this proteinase to Bacillus thuringiensis resistance. We proposed that insects lacking this critical gut proteinase have a selective advantage when feeding on Bacillus thuringiensis -treated plants. This poses a serious threat to the longevity of Bacillus thuringiensis transgenic crops. The results of this study were widely disseminated at meetings and published in the *Journal of Biological Chemistry* (Oppert et al., 1997c).

<u>Plan of Work FY98:</u> We plan to survey the proteinase patterns in laboratory strains of the Indianmeal moth and field strains of major crop pests feeding on Bacillus thuringiensis transgenic plants. This will estimate the extent to which insects can adapt to Bacillus thuringiensis toxins via proteinase-mediated mechanisms.

Publications:

Howard, R. W. Ontogenetic, reproductive, and nutritional effects on the cuticular hydrocarbons of the host-specific ectoparasitoid *Cephalonomia tarsalis* (Hymenoptera: Bethylidae). Annals Entomological Society of America. (In press)

Johnson, D. E., B. Oppert, and W. H. McGaughey. Spore coat protein synergizes *Bacillus thuringiensis* crystal toxicity for the Indianmeal moth (*Plodia interpunctella*). Curr. Microbiol. (In press)

McGaughey, W. H. and B. Oppert. Control of Agricultural Insect Pests and Vectors of Human Diseases. Proc. of the 2nd En Gedi Conference on Bacterial Control of Agricultural Insect Pests and Vectors of Human Diseases. (In review)

Mohammed, S. I., D. E. Johnson, and A. I. Aronson. 1996. Altered binding of the Cry1Ac toxin to larval membranes but not the toxin-binding protein in *Plodia interpunctella* selected for resistance to different *Bacillus thuringiensis* isolates. Appl. Environ. Microbiol. 62, 4168-4173.

Oppert, B., K. J. Kramer, R. W. Beeman, D. E. Johnson, and W. H. McGaughey. 1997. Proteinase-mediated resistance to *Bacillus thuringiensis* insecticidal toxins. J. Biol. Chem., 272: 23473-23476.

Oppert, B. and K. J. Kramer. Economical proteinase activity blot. BioTechniques. (In review)

Oppert, B., K. J. Kramer, and W. H. McGaughey. 1997. Insect resistance to *Bacillus thuringiensis* toxins, The Chemist, 74: 7-10.

Oppert, B., K. J. Kramer, and W. H. McGaughey. 1997. Rapid microplate assay of proteinase mixtures. BioTechniques, 23: 70-72.

Reeck, G., B. Oppert, M. Denton, M. Kanost, J. E. Baker, and K. J. Kramer. Book Chapter, Insect proteinases. *In* Proteases (Turk, V., ed.), Birkhauser, Basel, Switzerland. (In review)

Zhu, Y. C., B. Oppert, K. J. Kramer, W. H. McGaughey, and A. K. Dowdy. Comparison of cDNA sequences, mRNA expression levels, and genomic DNAs for trypsinogen-like enzyme in Bacillus thuringiensis -susceptible

Zhu, Y. C., B. Oppert, K. J. Kramer, W. H. McGaughey, and A. K. Dowdy. Comparison of cDNA sequences, mRNA expression levels, and genomic DNAs for trypsinogen-like enzyme in Bacillus thuringiensis -susceptible and Bacillus thuringiensis -resistant strains of *Plodia interpunctella*. Insect Mol. Biol. (In review)

Zhu, Y. C., B. Oppert, K. J. Kramer, W. H. McGaughey, and A. K. Dowdy. Characterization of a gene coding for a chymotrypsin-like proteinase from *Plodia interpunctella* strains with different gut proteinase activities. Insect Biochem. Mol. Biol. (In press)

RESEARCH PROGRESS AND PLANS

CRIS No. 5430-43000-016-00D

Title: Development of Physiological and Genetic Controls for Stored Product Insects

Main Objectives:

The goals of this project are to characterize physiological and genetic processes that can be manipulated for insect control purposes, identify inhibitory proteins and genetically modify cereal grains and entomopathogens with their genes, develop techniques for genetic manipulation of insects, devise genetic methods for managing pesticide resistance in insects and for enhancing the efficacy of beneficial insects. Specific objectives include identifying target sites in insect skeletal, gut and endocrine systems vulnerable to biopesticides; characterizing molting and digestive enzymes, identifying inhibitors whose genes are amenable to plant and microbial genetic engineering; evaluating recombinant seeds for resistance to insects and evaluating recombinant microbial pathogens for efficacy as biological control agents; identifying inhibitors of insect hormones, evaluating modes of action and efficacy as regulators of growth and physiological processes; characterizing cuticular components of pests and parasites, assessing functional responses to environmental stress; characterizing genetic mechanisms regulating reproduction, development and pesticide resistance of pest and beneficial insects; and developing techniques for genetically altering, manipulating and monitoring populations of pest and beneficial insects.

Investigators: **Karl J. Kramer, James E. Baker, Richard W. Beeman**

1. Specific Objective: Identify potential biopesticides and screen them for their ability to disrupt stored grain insect development and physiology.

Progress FY97: Indian meal moth digestive proteinases that activate the insecticidal protoxin of Bacillus thuringiensis (Bacillus thuringiensis) were characterized. A novel mechanism of proteinase-mediated insect resistance to Bacillus thuringiensis toxins was identified. cDNAs for chymotrypsinogen-like proteins from two strains of the Indian meal moth, one susceptible and one resistant to Bacillus thuringiensis , were isolated and characterized. Their genes were expressed in a similar manner and their encoded proteins were identical in amino acid sequence. Reviews on plant resistance to stored product insect pests,

insect proteinases, proteinase inhibitors and resistance of transgenic plants to insects were written.

Plan of Work FY98-99: Continue studies on the development of biopesticides and insect growth regulators that disrupt insect gut and cuticle physiology for control of insect pests in food and stored products.

2. Specific objective: Characterize reactants, intermediates, products and enzymes involved in insect cuticle sclerotization and immune responses, which might be targets for novel insect selective control agents.

Progress FY97: Several enzymes, metabolites, proteins, and cross-linked structures responsible for cuticle sclerotization in moths and flies were characterized. A method for distinguishing regioselective adducts of catecholamines and amino acids found in insect cuticles was developed. Mechanisms for reactions between cuticle cross-linking agents and protein model nucleophiles were elucidated. A novel antibacterial adduct was identified in the hemolymph of immuninized insects. A review on the use of solid state nuclear magnetic resonance for studies of intractable biological samples was written.

Plan of Work FY98-99: Continue to identify and characterize insect-specific reactions that occur during cuticle sclerotization and immune responses, which could serve as targets for new insect growth regulators and biopesticides for use in protecting food and stored products against insect pests.

3. Specific Objective: Clone insect chitinolytic enzyme genes and study their molecular biology to determine how insects regulate chitin degradation during molting. Use chitinolytic enzymes as biopesticides to help control pest insects by manipulating chitinolytic enzyme genes in transgenic plants and microbial entomopathogens.

Progress FY97: A genomic clone and a cDNA for insect chitinase and a ß-N acetylglucosaminidase and a recombinant insect chitinase from transgenic tobacco were isolated and characterized. Amendments and declarations for foreign patent application for recombinant insect chitinase and its use as a biocide were filed. Reviews on the use of chitinolytic enzymes for insect control and the molecular biology of insect chitinases were written.

Plan of Work FY98-99: Continue to develop insect chitinolytic enzymes as biopesticides for use in protecting food and stored products from insect pests and microbial pathogens.

4. <u>Specific Objective:</u> Initiate studies to characterize the biochemical mechanism(s) of malathion resistance in the parasitoid *Anisopteromalus calandrae.*

<u>Progress FY97</u>: Esterase activity in adults of *A. calandrae* was measured with four substrates. There were no consistent differences among Vmax/Km ratios for these substrates and a number of their analogs between malathion-resistant (R) and malathion-susceptible (S) strains of *A. calandrae.* Malathion carboxyleesterase (MCE) was 10-30-fold more active in the R strain compared with the S strain. A number of biochemical properties of MCE and the general esterases were determined.

<u>Plan of Work FY98</u>: Continue to evaluate the biochemical mechanism of malathion resistance in the R strain. Develop protocols to purify the MCE from the R strain.

5. <u>Specific Objective:</u> Characterize molecular genetics of malathion resistance in *Anisopteromalus calandrae.*

<u>Progress FY97</u>: Two DNA markers specific for a malathion-resistant strain of *A. calandrae* were developed. A single base substitution found in partial cDNA sequences in R and S strains was confirmed by PASA. The substitution was inherited in a strict Mendelian manner and was linked to resistance.

<u>Plan of Work FY98</u>: Continue studies on molecular mechanisms of resistance.

6. <u>Specific Objective:</u> Evaluate potential of automated NIR-SKCS to rapidly and accurately determine hidden insect infestations in wheat kernels.

<u>Progress FY97</u>: The integrated NIR-SKCS can rapidly and accurately detect insect infestations inside wheat kernels. Detection of hidden insects was not affected by wheat class, moisture content of wheat, or protein content of different wheat cultivars but was affected by larval size.

<u>Plan of Work FY98</u>: Continue evaluation of NIR-SKCS for detection and analysis of insects in grain.

7. <u>Specific Objective:</u> Compare electrophoretic properties of esterases and MCE in resistant and susceptible strains of *A. calandrae.*

<u>Plan of Work FY98</u>: Esterases and MCE will be analyzed with native PAGE, SDS, and IEF gels. Protein bands will be characterized with the use of specific substrates and inhibitors.

8. <u>Specific Objective:</u> Characterize malathion resistance in Dickinson strain of *Bracon hebetor*.

<u>Plan of Work FY98</u>: Compare biochemical detoxification systems in R and S strains of *B. hebetor*.

9. <u>Specific Objective</u>: Develop methodology for genetic manipulation of pest insects.

<u>Progress FY97</u>: We tested a lepidopteran transposable element for ability to function as a gene transfer agent in flour beetles. Candidate transformants could not be confirmed in subsequent tests. A new approach was initiated, involving use of eye-color selection for recognition of transformants. Most of the Tribolium white-eye gene was cloned using 3' RACE (Rapid Amplification of cDNA Ends).

<u>Plan of Work FY98</u>: Work is underway to finish cloning the white-eye gene and to develop white-based gene transfer vectors. Expected benefits include the ability to infect pest species with insect control genes (viruses, lethal genes, transposons), transfer of pesticide resistance genes to parasitoids, and transfer of deleterious traits (such as disease susceptibility, pesticide susceptibility and cold intolerance) to pest species.

10. <u>Specific Objective</u>: Characterize insect resistance to grain protectants.

<u>Progress FY97</u>: A cytochrome P450-associated gene for pyrethroid resistance was mapped at high resolution on the 9th linkage group.

<u>Plan of Work FY98</u>: A group of esterase genes suspected to be involved in malathion resistance will be mapped in Tribolium, and individual genes directly involved in the resistance will be identified.

11. <u>Specific Objective</u>: Harness natural insect control genes.

<u>Progress FY97</u>: Map-based cloning of a naturally-occurring killer gene M1 was completed. A whole-genome molecular map was refined, and DNA markers closely linked to the killer gene were identified, cloned and sequenced, and converted to sequence-tagged-sites.

<u>Plan of Work FY98</u>: We will continue to pursue the map-based cloning of other killer genes using the technique of Genetically Directed Representational Difference Analysis (GDRDA). Understanding the lethal mechanism of this unusual gene could lead to new approaches for pest control.

Publications:

Baker, J. E., J. Perez Mendoza, and R. W. Beeman. Inheritance of malathion resistance in the parasitoid *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae). J. Econ. Entomol. 90:304-308.

Baker, J. E., J. Perez Mendoza, and R. W. Beeman. 1996. Multiple mating potential in a pteromalid wasp determined by using an insecticide resistance marker. J. Kansas Entomol. Soc. (In review)

Beeman, R. W. and D. M. Stauth. 1997. Rapid cloning of insect transposon insertion junctions using "universal" PCR. Ins. Molec. Biol. 6:83-88.

Choi, H., K. Choi, K. J., Kramer, and S. Muthukrishnan. 1997. Isolation and characterization of a genomic clone for the gene of an insect molting enzyme, chitinase. Insect Biochem. Molec. Biol. 27:37-47.

Ding, X., B. Gopalakrishnan, L. B. Johnson, F. F. White, X. Wang, T. W. Morgan, K. J. Kramer, and S. Muthukrishnan. Resistance to tobacco budworm feeding by transgenic plants expressing an insect chitinase gene. Transgenic Res. (In press)

Dowell, F. E., J. E. Throne, and J. E. Baker. Automated nondestructive detection of internal insect infestation in wheat kernels using near-infrared reflectance spectroscopy. J. Econ. Entomol. (In review)

Dowell, F. E., J. E. Throne, and J. E. Baker. 1997. Detection of hidden insects in grain by automated NIR reflectance spectrophotometry. Ann. Internat. Conf. Methyl Bromide Alternatives and Emissions Reductions, Nov. 3-5, 1997, San Diego, CA, pp. 108-1-108-2.

Dowell, F. E., J. L. Steele, C. R. Martin, J. E. Throne, J. E. Baker, and S. R. Delwiche. Automated single wheat kernel quality measurement using near-infrared reflectance and the single kernel characterization system. Proc. Cereal Chemistry. (In press)

Huang, X., R. Xu, M. D. Hawley, and K. J. Kramer. Model insect cuticle sclerotization: reactions of catecholamine quinones with the nitrogen-centered nucleophiles imidazole and N-acetylhistidine. Bioorg. Chem. (In press)

Huang, X., R. Xu, M. D. Hawley, T. L. Hopkins, and K. J. Kramer. Electrochemical oxidation of N-acyldopamines and subsequent regioselective reactions with the sulfur-centered nucleophiles N-acetylcysteine and thiourea. Arch. Biochem. Biophys. (In press)

Kramer, K. J. 1996. ARS biotechnology research in plant resistance to stored product insect pests. Proceedings First Joint Meeting U.S. - Japan Natural Resources Protein and Toxic Microorganisms Panels (A. Pavlath, ed.), FF1-FF13.

Kramer, K. J., S. Muthukrishnan, L. Johnson, and F. White. 1997. Book Chapter, Chitinases for insect control. *In* Advances in Insect Control: The Role of Transgenic Plants. (N. Carozzi and M. Koziel, eds.), Taylor and Francis Publ., Bristol, PA. pp. 185-193.

Kramer, K. J., and S. Muthukrishnan. Insect chitinases: molecular biology and potential use as biopesticides. Insect Biochem. Molec. Biol. (In press)

Kramer, K. J., T. L. Hopkins, and J. Schaefer. Analysis of intractable biological samples by solids NMR. In Fate of N-Containing Macromolecules in the Biosphere and Geosphere. (Stankiewicz, A., ed.), Amer. Chem. Soc., Adv. Chem. Series. (In press)

Merritt, M. E., A. M. Christensen, K. J. Kramer, T. L. Hopkins, and J. Schaefer. 1996. Detection of inter-catechol-protein cross-links in insect cuticle by solid state carbon and nitrogen-15 NMR. J. Amer. Chem. Soc. 118, 11278-11282.

Oppert, B. and K. J. Kramer. Economical proteinase activity blot. BioTechniques. (In review)

Oppert, B., K. J. Kramer, R. W. Beeman, D. E. Johnson, and W. H. McGaughey. 1997. Proteinase-mediated insect resistance to *Bacillus thuringiensis* toxins. J. Biol. Chem. 272, 23473-23476.

Oppert, B., K. J. Kramer, and W. H. McGaughey. 1997. Insect resistance to *Bacillus thuringiensis* toxins, The Chemist, 74: 7-10.

Oppert, B., K. J. Kramer, and W. H. McGaughey. 1997. Rapid microplate assay of proteinase mixtures. BioTechniques, 23: 70-72.

Reeck, G., B. Oppert, M. Denton, M. Kanost, J. E. Baker, and K. J. Kramer. Insect proteinases. *In* Proteases (Turk, V., ed.), Birkhauser, Basel, Switzerland. (In review)

Reeck, G., K. J. Kramer, J. E. Baker, M. Kanost, J. Fabrick, and C. Behnke. 1997. Proteinase inhibitors and resistance of transgenic plants to insects. *In* Advances in Insect Control: The Role of Transgenic Plants. (N. Carozzi and M. Koziel, eds.), Taylor and Francis Publ., Bristol, PA, pp. 157-183.

Turecek, F., J. Kerwin, R. Xu, and K. J. Kramer. Distinction of N-Substituted Histidines by Electrospray Ionization Mass Spectrometry. J. Mass Spectrometry. (In press)

Wappner, P., K. J. Kramer, F. Manso, T. L. Hopkins, and L. A. Quesada-Allué. 1996. N-ß-Alanyldopamine metabolism for puparial tanning in wild-type and mutant niger strains of the Mediterranean fruit fly, *Ceratitis capitata.* Insect Biochem. Molec. Biol. 26, 585-592.

Wang, X., X. Ding, B. Gopalakrishnan, T. D. Morgan, L. Johnson, F. White, S. Muthukrishnan, and K. J. Kramer. 1996. Characterization of a 46 kDa insect chitinase from transgenic tobacco. Insect Biochem. Molec. Biol. 26, 1055-1064.

Xu, R., X. Huang, T. L. Hopkins, and K. J. Kramer. 1997. Catecholamine and histidyl protein cross-linked structures in sclerotized insect cuticle. Insect Biochem. Molec. Biol. 27, 101-108.

Zhu, Y. C., B. Oppert, K. J. Kramer, W. H. McGaughey, and A. K. Dowdy. Comparison of cDNA sequences, mRNA expression levels, and genomic DNAs for trypsinogen-like enzyme in Bacillus thuringiensis -susceptible and Bacillus thuringiensis -resistant strains of Plodia interpunctella, Insect Mol. Biol. (In review)

Zhu, Y. C., B. Oppert, K. J. Kramer, W. H. McGaughey, and A. K. Dowdy. CDNAs for a chymotrypsinogen-like protein from two strains of *Plodia Interpunctella.* Insect Biochem. Molec. Biol. (In press)

Zhu, Y. C., A. K. Dowdy, and J. E. Baker. Genomic DNA polymorphisms in malathion-resistant and -susceptible strains of the parasitoid *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae). J. Econ. Entomol. (In review)

Zhu, Y. C., A. K. Dowdy, and J. E. Baker. PCR confirmation of single-base substitution on an esterase gene and linkage to malathion resistance in the parasitoid *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae). (In Review)

Patent:

Kramer, K. J., S. Muthukrishnan, H. K. Choi, L. Corpuz, and B. Gopalakrishnan. Recombinant chitinase and use thereof as a biocide. International Patent Application #PCT/US96/14440.

RESEARCH PROGRESS AND PLANS

CRIS No. 5430-43000-017-00D

Title: Monitoring and control strategies for stored-product insects

Main objectives:

The overall objective of this CRIS is to develop monitoring and control strategies for stored-product insect pests in and around storage and processing facilities. These studies will lead to the development and improvement of genetic, semiochemical and physical techniques to monitor insect behavior, estimate population densities, and determine control thresholds and timing of management actions. Selected control tactics will be evaluated for integration into ecologically-compatible pest management programs. Insect resistant packaging and barriers will be developed to prevent infestation of stored products. The acquired knowledge will lay the ground work necessary to evaluate the efficacy of current practices and to develop ecologically-sound insect pest management programs.

Investigators: **Michael A. Mullen, Franklin H. Arthur, Alan K. Dowdy**

1. Specific Objective: Development of a disposable pheromone baited trap for stored-product Coleoptera.

Progress FY 97: The trap was made from a 15 dram snap cap vial with openings for the insects to enter and baited with a pheromone lure. A food oil was used as a short range attractant and a killing agent. Laboratory comparisons with the FLIT TRAK M^2 that showed the vial trap to be about equal in effectiveness for capturing adult red flour beetles. This trap was designed to be used in areas where the FLIT TRAK M^2 would not be suitable. Research has been completed and a manuscript has been submitted to J. Entomol. Sci.

Plan of Work for FY 98: None

2. Specific Objective: Evaluation of pheromone formulations for the sawtoothed grain beetle.

Progress FY 97: Project on hold until new pheromone formulations are received.

Plan of Work for FY98: None planned.

3. **Specific Objective:** Redesign the FLIT-TRAK M^2 to improve the rate of catch and ease of counting trapped insects.

Progress FY 97: A redesign of the trap was tested. The lower profile trap was developed as well as a one-piece design to make the trap easier to use. Recommendations were made to the design engineer to alter the design without impacting its effectiveness.

Plan of Work for FY 98: As new prototypes become available testing will be continued. A wall mounted trap using the FLIT-TRAK M^2 is being developed.

4.**Specific Objective:** A new pheromone trap will be designed to use in specialized areas, such as grocery stores, so that they will remain out of the view of the public.

Progress FY 97: An under the shelf trap was designed and tested in grocery stores. The trap was found to be as effective as the Pherocon II.

Plan of Work FY 98: A manuscript is being prepared and the marketing of the trap has been discussed with a trap manufacturer.

5. **Specific Objective:** Use of pheromone traps as a dispersal mechanism for granulosis virus.

Progress FY 97: No transmission of the virus using pheromone baited traps was demonstrated and the project has been terminated.

6. **Specific Objective:** Improvement of seals and closures on commercial packaging to reduce infestation by stored-product insects.

Progress FY 97: Cooperation with food processing companies was expanded. Tests for several companies were conducted. A new package was developed and is now undergoing testing.

Plan of Work for FY 98: Testing of packages will be continued and recommendations will be made to the manufacturers to improve insect resistance.

7. **Specific Objective:** Test chemical odor neutralizers for use in insect resistant packaging.

Progress FY97: At the request of the manufacturer, testing was discontinued.

Plan of Work for FY 98: Test on odor neutralizers has been discontinued. Testing of the material will continue.

8. **Specific Objective:** Test, evaluate, and make recommendations on several new repellant compounds for use in insect resistant packaging.

Progress FY 97: These compounds were tested on breakfast cereal, cat food, and baby cereal. The company has applied for FDA approval. Other compounds were screened for effectiveness as a repellent and two were identified as effective in laboratory tests. Longevity tests are still being conducted and one compound is still effective after five months.

Plan of Work FY 98: New compounds will be tested as they become available. Some compounds will be tested in large scale package tests.

9. **Specific Objective:** Evaluation of residual insecticides on different flooring surfaces.

Progress FY97: Confused flour beetles were exposed for short time intervals (0.5 to 4 hours) on concrete treated with 9.5 and 19.0 g of 20% cyfluthrin wettable powder per 1,000 ft^2. At least 90% of the beetles were killed at both application rates when exposed at all time intervals during a 12-week residual testing period. After 12 weeks, longer exposure intervals were required to achieve the same level of mortality. When wandering-phase Indianmeal moth larvae were exposed under the same conditions, these short exposure intervals did not prevent pupation and adult emergence.

Plan of work FY98: Cooperate with industry to conduct evaluations of new chemicals. Conduct additional research with established chemicals.

10. **Specific Objective:** Determine residual efficacy of new insecticides as grain protectants.

Progress FY97: Timed exposure studies have been concluded for cyfluthrin E.C. applied to stored wheat. Work is still in progress on a cyfluthrin encapsulated formulation that is being evaluated as a grain protectant.

Plan of work FY98: Analyze data and continue with experiments that are in progress.

11. **Specific Objective:** Determine the potential for the expanded use of aeration on stored grains.

Progress FY97: A maize weevil population growth model was integrated with temperature data to predict insect development at different aeration management strategies for corn stored in the southeastern United States.

Plan of work FY98: Continue cooperative efforts regarding insect population models and how they could be used to develop aeration plans.

12. Specific Objective: Evaluate microbial pathogens as protectants of stored grains.

Progress FY97: Cooperated with several companies to test microbial and fungal pathogens to control stored-product insects. Results have been inconclusive regarding efficacy and new testing methods must be developed.

Plan of work FY98: Refine testing methodologies and continue with research regarding promising candidate microbial and fungal pathogens.

13. Specific Objective: Determine sources of insect infestation along marketing channels by using DNA fingerprinting technology.

Progress FY97: Preliminary analysis of lesser grain borer was conducted to identify genetic markers that can differentiate individual insects into population groups.

Plan of Work FY98: Work will be expanded this year in conjunction with an area-wide pest management program.

14. Specific Objective: Examine DNA fingerprints to detect and monitor for insecticide resistance.

Progress FY97: Screening of the Indianmeal moth for genetic fingerprints linked to resistance to the bacterial insecticide *Bacillus thuringiensis* was conducted. Characterized the expression of messenger RNA that codes for aminopeptidase in Indianmeal moth was conducted. Screened *Anisopteromalus calandrae,* a parasitoid of the rice weevil, for genetic fingerprints linked to resistance to Malathion. Characterized the expression of messenger RNA that codes for a carboxyesterase in *A. calandrae.* Acquired lesser grain borer populations that have known resistance to phosphine.

Plan of Work FY98: Research on using DNA fingerprints to detect and monitor insecticide resistance in the Indianmeal moth, lesser grain borer and flat/rusty grain beetle will continue. This may eventually lead to a quick test to be used in field situations.

15. Specific Objective: Evaluate the distribution of heat in cereal grain processing plants as an alternative to methyl bromide fumigation for insect control.

Progress FY97: Heat sterilization procedures for insect management were monitored in three processing facilities. The distribution and stratification of heat has an impact on the effectiveness of this method for insect control. Most areas examined would benefit from the addition of fans to improve air circulation that will result in more uniform application of heat.

Plan of Work FY98: Additional heat sterilization procedures will be monitored and the impact on airflow on heat distribution will be evaluated. This technology has the potential of being an effective alternative to methyl bromide fumigation in processing plants.

16. Specific Objective: Evaluate the combined use of heat and diatomaceous earth on insect mortality.

Progress FY97: Laboratory tests indicate that the use of diatomaceous earth in combination with heat has the potential of reducing the temperature or time necessary to effect adequate insect management. When red flour beetles were treated with diatomaceous earth and exposed to 50°C for 15 minutes, mortality was comparable to beetles that were heated to 50°C for 30 minutes but not treated with diatomaceous earth.

Plan of Work FY98: Time by temperature by humidity interactions will be examined for combined use of heat and diatomaceous earth. Different formulations of diatomaceous earth will also be tested in combination with heat.

17. Specific Objective: Evaluate the combined use of heat and cyfluthrin on insect mortality.

Progress FY97: Concrete panels treated with cyfluthrin were heated to 50°C for up to 16 hours to determine if there was any reduction in efficacy after heating. Compared to unheated panels, there was no reduction in confused flour beetle mortality when the cyfluthrin panels were heated. It appears that the efficacy of this compound will not be adversely affected if used in processing plants that use heat sterilization as part of their insect management program.

Plan of Work FY98: Additional chemical crack and crevice, and surface treatments will be evaluated after exposure to high temperature to determine their value in integrated pest management programs that use heat sterilization.

18. <u>Specific Objective</u>: Spatial analysis of stored-product insect populations in processing plants, warehouses and retail stores.

<u>Progress FY97</u>: The locations of stored-product insect infestations were identified using spatial analysis methods. Sources of infestations previously unknown to facility managers were identified. Insect infestations appear to be related to equipment design problems, inadequate sanitation and poor stock rotation.

<u>Plan of Work FY98</u>: Expanded work in food warehouses will be conducted pending availability of funds.

Publications:

Arthur, F. H. 1997. Differential effectiveness of deltamethrin dust on wood, concrete, and tile surfaces against three stored-product beetles. J. Stor. Prod. Res. 33: 167-173.

Arthur, F. H. 1997. Residual susceptibility of *Plodia interpunctella* to deltamethrin dust: effect of concentration and time of exposure. J.Stor. Prod. Res. (In Press).

Dowdy, A. K. And M. A. Mullen. Multiple stored-product insect pheromone use in pitfall traps. J. Stor. Prod. Res. (In press)

Dowdy, A. K. And W. H. McGaughey. Stored-product insect activity outside of grain masses in commercial elevators in the Midwestern United States. J. Stor. Prod. Res. (In press)

Fields, P., A. K. Dowdy, and M. Marcotte. 1997. Structural Pest Control: The use of an enhanced diatomaceous earth product combined with heat treatment for the control of insect pests in food processing plants. Leadership in the development of methyl bromide alternatives. Environmental Bureau, Agriculture and Agri-Food Canada and United States Department of Agriculture, pp. 1-25.

Mullen, M. A., E. P. Wileyto, and F. H. Arthur. Influence of trap design and location on the capture of *Plodia interpunctella* (Indianmeal moth)(Lepidoptera: Pyralidae) in a controlled release study. J. Stored Prod. Res. (In press)

Mullen, M. A., 1997. Keeping Bugs at Bay. J. Feed Management and Marketing. 48: 29-33.

Zettler, J. L. and F. H. Arthur. Dose-response on red flour beetle, *Tribolium castaneum* (Herbst) and the confused flour beetle, *Tribolium castaneum* Jacqueline duVal, collected from flour mills in the United States. J. Econ. Entomol. (In press)

Zhu, Y. C., B. Oppert, K. J. Kramer, W. H. McGaughey, and A. K. Dowdy. CDNAs for a chymotrypsinogen-like protein from two *Plodia interpunctella* strains with different susceptibilities to *Bacillus thuringiensis* toxin. Ins. Biochem. & Molec. Biol. (In press)

Publications

Arthur, F. H. 1997. Differential effectiveness of deltamethrin dust on wood, concrete, and tile surfaces against three stored-product beetles. J. Stor. Prod. Res. 33: 167-173.

Arthur, F. H. 1997. Rapid knockdown capability of beetles exposed to cyfluthrin: effect of concentration and time of exposure. J. Stor. Prod. Res. (In press).

Dowdy, A. K. And M. A. Mullen. Multiple stored product insect pheromone use in pitfall traps. J. Stor. Prod. Res. (In press)

Dowdy, A. K. And W. H. McGaughey. Stored-product insect activity outside of grain masses in commercial elevators in the Midwestern United States. J. Stor. Prod. Res. (In press)

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